UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH ADMINISTRATION



Bureau of Agricultural and Industrial Chemistry
Agricultural Research Center
Beltsville, Maryland

September 15, 1950

TO: G. W. Irving, Jr., Asst. Chief of Bureau
AIC, South Building, Washington, D. C.

FROM: T. D. Fontaine, Head, Biologically Active Compounds Div., ARC, Beltsville, Maryland

SUBJECT: Report of trip to the University of New Brunswick, Fredericton, New Brunswick, August 21-25, 1950 for the purpose of attending the Second Summer Seminar in the Chemistry of Natural Products

Summary: - The following papers presented at the meeting are abstracted:

Biosynthesis of Isoquinolines, by R. H. Manske, Dominicon Rubber Co.

Annotinine, by P. Meister, National Research Council of Canada

Thermopine, by L. Marion, National Research Council of Canada

Steroid Secondary Amines, by F. C. Uhle, Harvard University.

This paper was of particular interest because he reported the partial synthesis of tomatidine.

The Chemical Nature of Fatty Acids of Bacterial Origin, by Klaus Hofmann, University of Pittsburgh.

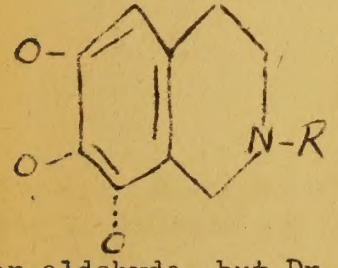
On the Mechanism of Oxidation of some Heterocyclic Systems, by B. Witkop, Harvard University (Dr. Witkop will soon report to work at the Heart Institute, National Institutes of Health, Bethesda, Maryland)

The Constitution of Cinchonamine and of Quinamine, by W. I. Taylor, National Research Council of Canada

Report:

Biosynthesis of Isoquinolines, by R. H. Manske, Dominion Rubber Company.

All isoquinoline alkaloids found in plants have the tetrahydro structure,

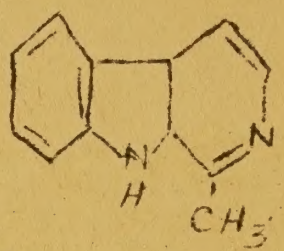



where R = H or $CH_3$ and O = OH, $OCH_3$, or $CH_2{<}O{>}O$. It is believed that the isoquinoline alkaloids, such as benzylisoquinoline, aporphines, protoberberine, protopine, chelidonine, phthalide-isoquinoline, and culanine types, are synthesized by the plant from amino acids. The ring closure of amino acid to form the isoquinoline structure is believed to take place by a condensation with an aldehyde, but Dr. Manske doubts that formaldehyde occurs in plants and thinks glyoxylic acid is a more likely agent. Further, it is believed that benzylisoquinoline is the first step in the synthesis of all other

isoquinolines. In the synthesis of these compounds benzylisoquinoline is used up almost completely.

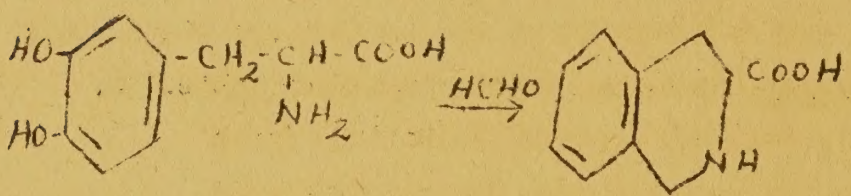
The first synthesis of an alkaloid from an amino acid was by the following reaction:

Tryptohan acetaldehyde →

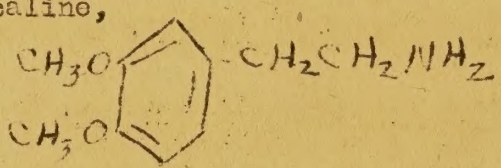


This alkaloid (harman) was later isolated from a plant.

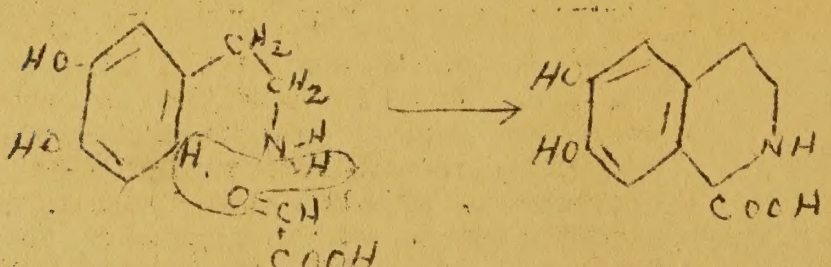
Robinson postulated that the synthesis of the isoquinoline took place as follows:



with the subsequent removal of the carboxyl. Dr. Manske does not believe that Robinson's postulate is correct and suggests another possible method. It is known that mescaline,



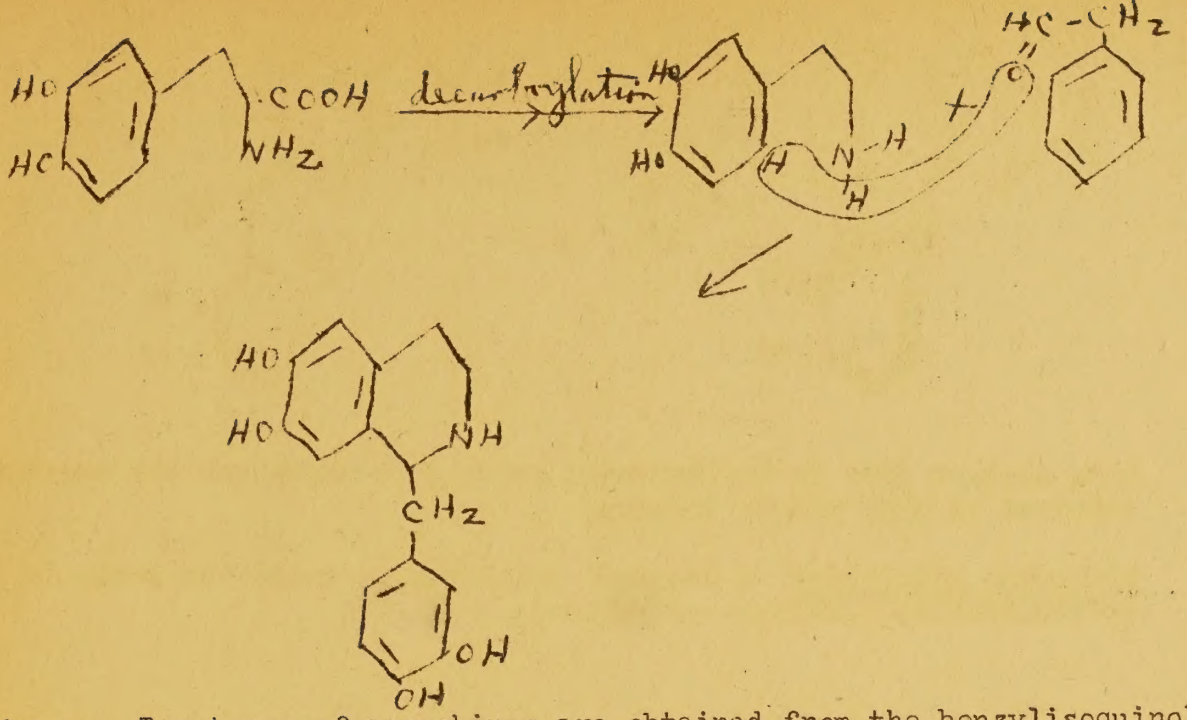
is a base which accompanies alkaloids found in the cactus family. It appears that the first step in the synthesis of the isoquinoline is the decarboxylation of the amino acid to the amine, followed by condensation with glyoxylic acid as follows:



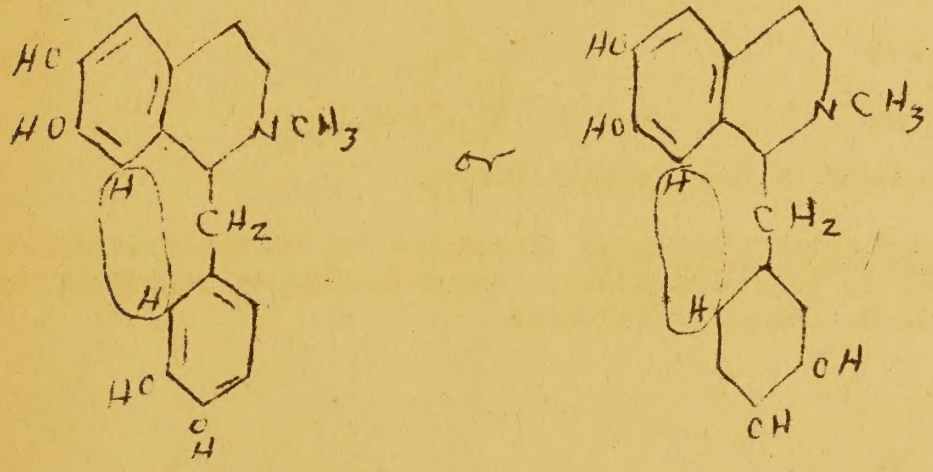
with a subsequent removal of the carboxyl groups. He admitted, however, that he knew of no biological systems which actually was capable of removing the carboxyl group.

Benzylisoquinoline alkaloids: The Papavaraceae are the only plants which produce the alkaloid primarily by the following supposed reaction in the

plant.

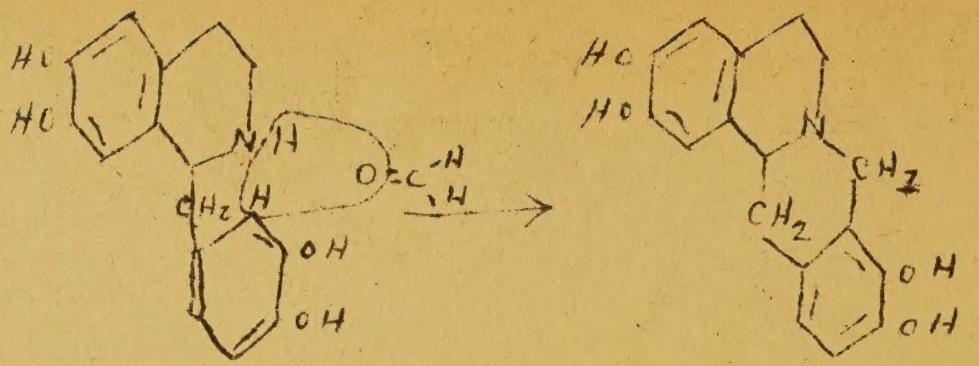



Aporphines: - Two types of aporphines are obtained from the benzylisoquinoline alkaloid because ring closure can take place by biological oxidation either ortho or para to the hydroxyl group, thus



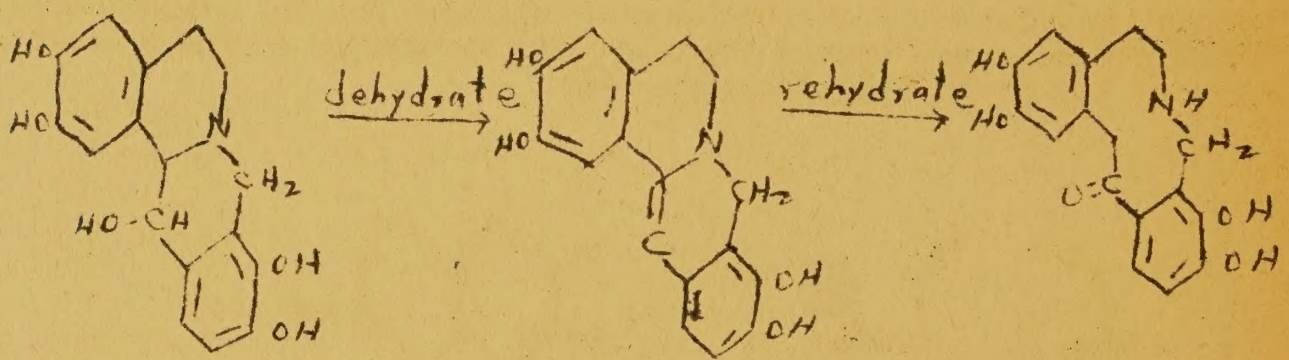

It is of interest that plants are unable to effect ring closure meta to a hydroxyl with the isoquinoline structure.

Protoberberine Alkaloids: Dr. Manske used formaldehyde only to indicate the type of condensation which may occur.



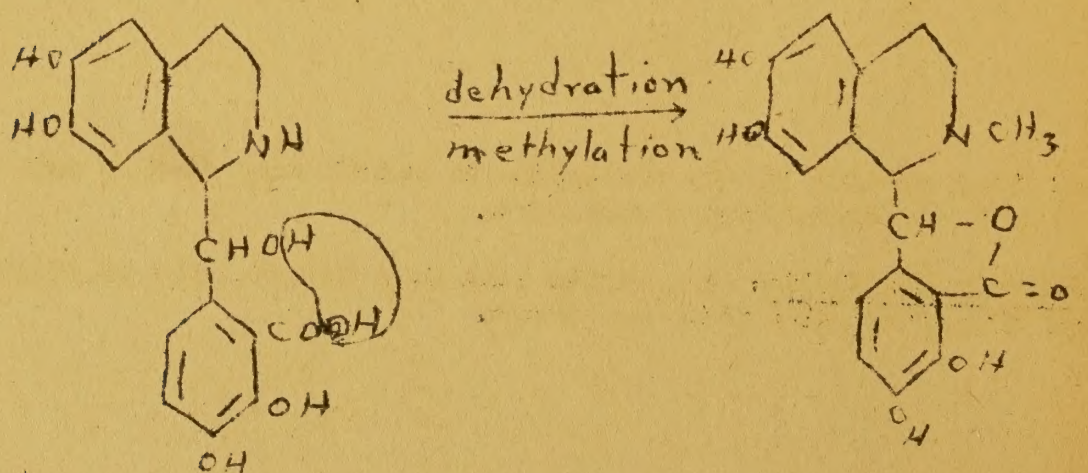
Ring closure para to the hydroxyl group does occur but the compound is obtained in only minute amounts.

Protopine Alkaloids: A possible synthesis of these alkaloids is from protoberberine alkaloids as follows:




A $CH_3$ is introduced on the nitrogen later.

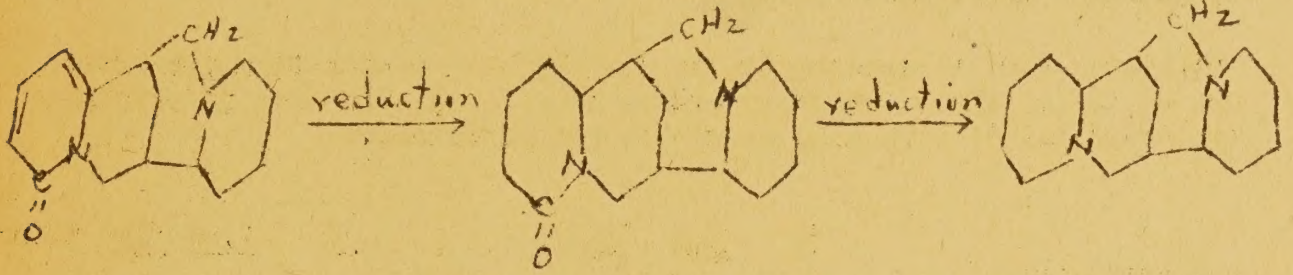
Phthalide-isoquinoline Alkaloids: Protopine and phthalide-isoquinoline alkaloids occur in plants together. The phthalide-isoquinoline alkaloids are believed to be formed as follows:



Annotinine, by P. Meister, National Research Council of Canada. Annotinine, $C_{16}H_{21}O_3N$, is the chief alkaloid obtained from Lycopodium annotinum L. This was a progress report since the structure of this material has not been elucidated. The following is known about the compound: Forms a methiodide indicating a tertiary nitrogen, one C-methyl group, two active hydrogens, no ketonic group, no unsaturation, six membered ring, possibly a $\gamma$-lactone, ether linkage. The claim that the compound contained a $\delta$-lactone was on the basis of infrared data. It was claimed that they could differentiate between a $\gamma$-lactone which absorbs at 1776 wave numbers as compared to a $\beta$-lactone which absorbs at 1730.

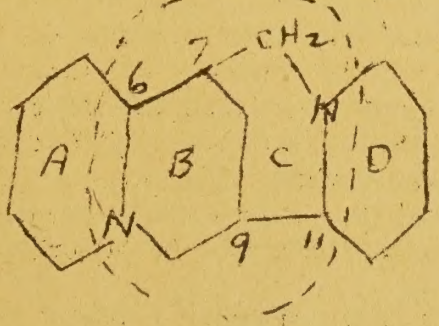
Thermopsine, by Leo Marion, National Research Council of Canada.

Thermopsine, $C_{15}H_{20}N_2O$, is an isomer of anagyrine. It has been established that

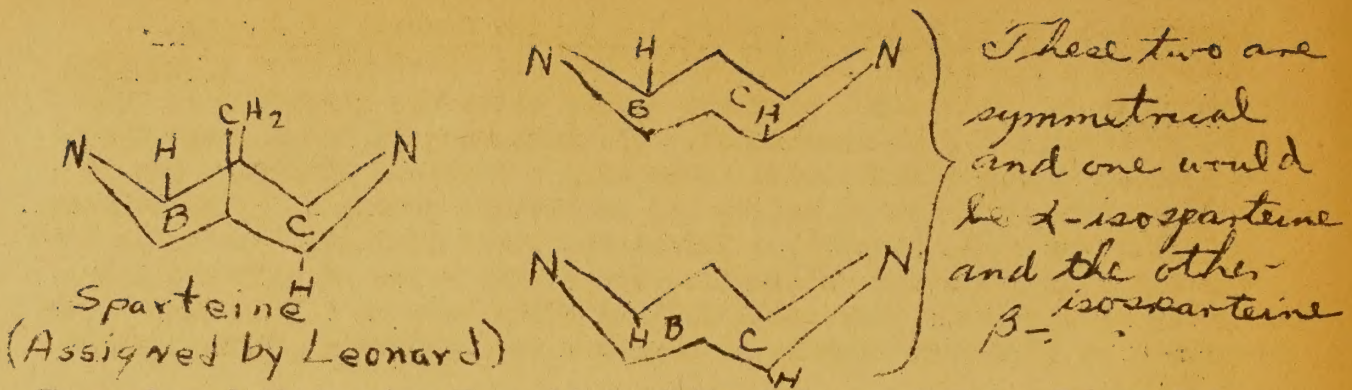



l-anagyrine l-lupanine d-sparteine

The $\alpha$-pyridone structure for thermopsine was established by infrared investigations. A clue to its structure was also given by catalytic hydrogenation. Thus, catalytic hydrogenation in neutral solution gave l-tetrahydrothermopsine, $C_{15}H_{24}N_2O$, an isomer of l-lupanine. Total reduction gave an isomer, $C_{15}H_{26}N_2.H_2O$, of sparteine, which was finally obtained free of water after many distillations. The melting point of the hydrate was 97-114°C and of the base 65°C. The problem was then to explain what the difference between anagyrine and thermopsine could be. This explanation was made on the basis of the spatial relationship of these compounds. The portion of the molecule in the dotted position




(rings B and C) was considered and may be shown as follows (the chair form has been used for illustration purposes only).




Thus, from l-thermopine they obtained the d-isosparteine. To complete the picture they needed to obtain d-thermopsine, which they did from Lupinus caudatis (unidentified), and completely reduced it. Upon reduction of d-thermopaine they obtained l-α-isosparteine. Therefore, the only difference between anagyrine and thermopsine is a spatial difference in rings B and C.

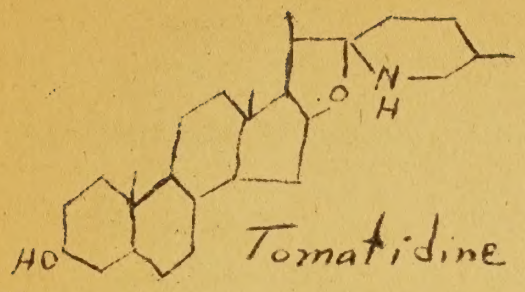
This gave them an opportunity to clarify some of the older literature. On the basis of 4 asymmetric carbon atoms at 6, 7, 9 and 11 positions the theoretical number of racemates was determined.

| | 6 | 7,9 | 11 | 6 | 7,9 | 11 | No. Racemates |
|---|---|---|---|---|---|---|---|
| Anagyrine type $C_{15}H_{20}N_2O$ | Blank | - Up | - Up | Blank | - Up | - Up | 2 |
| Lupanine type $C_{15}H_{24}N_2O$ | up-up-up | down-up-up | | up-up-down | down-up-down | | 4 |
| Sparteine $C_{15}H_{26}N_2$ | up-up-up | down-up-up | | | down-up-down | | 3 |

There are now in the literature more alkaloids of the $C_{15}H_{20}N_2O$ formula and more of $C_{15}H_{24}N_2O$ formula than allowed. Some of these compounds have been eliminated, such as hexalupine (Dr. Couch) which is the same as the thermopsine, and monolupine (Dr. Couch) which is the same as anagyrine. One alkaloid, sophoranine, has not yet been identified as either anagyrine or thermopsine. Only four alkaloids with the $C_{15}H_{24}N_2O$ formula are allowed but there are five recorded in the literature, lupanine, α-isolupine, monalupine, matrine, and sophorcarpine, so it would be expected that one of the last three listed is identical with one of the other two.

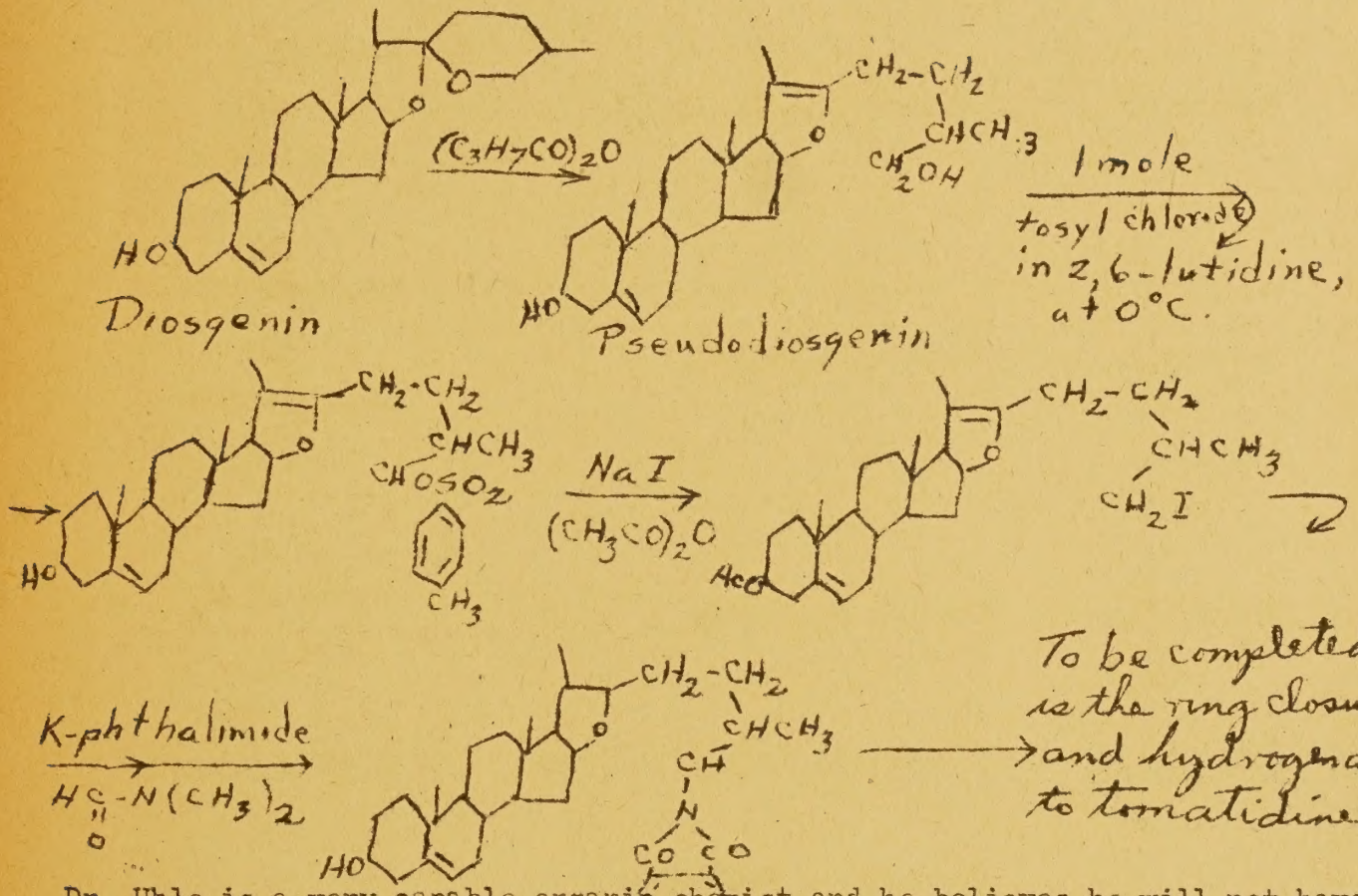
Steroid Secondary Amines by F. C. Uhle, Harvard Medical School.

This paper was the most important from the standpoint of the work of our division. Dr. Uhle has taken the proposed formula for tomatidine,



Tomatidine

, and is attempting a synthesis as follows:




Dr. Uhle is a very capable organic chemist and he believes he will not have any difficulty in closing the ring. He has completed the synthesis up to that point and will send me a sample of his final product when the synthesis is completed.

Dr. Uhle gave a very excellent review of the Veratrum and Solanum alkaloids which include secondary and tertiary amino bases and may be summarized as follows:

| Natural Product | Base | Non-nitrogen constituents |
|---|---|---|
| Protoveratrine | Protoverine, $C_{27}H_{43}NO_9$ | + $CH_3COOH$; $CH_3CH_2CH(CH_3)COOH$; $CH_3CH_2C(CH_3)_2COOH$ |

| Natural Product | Base | | Non-nitrogen constituents |
|---|---|---|---|
| Germitrine | Germine, $C_{27}H_{43}NO_8$ | + | $CH_3COOH$; $CH_3-CH_2-CH(CH_3)COOH$; $CH_3CH_2-C(CH_3)_2-COOH$ |
| Germidine | Germine, $C_{27}H_{43}NO_8$ | + | $CH_3COCH$; $CH_3CH_2CH(CH_3)COOH$ |
| Germerine | Germine, $C_{27}H_{43}NO_8$ | + | $CH_3CH_2CH(CH_3)COOH$; $CH_3CH_2C(CH_3)_2-COOH$ |
| Veratridine | Cevine, $C_{27}H_{43}NO_8$ | + | 3,4-dimethoxybenzoic acid |
| Cevadine | Cevine, $C_{27}H_{43}NO_8$ | + | $CH_3CH=C(CH_3)COOH$ |
| | Zygadenine, $C_{27}H_{43}NO_7$ | | |
| | *Unnamed, $C_{27}H_{41}NO_4$ | | |
| Pseudojervine | *Jervine, $C_{27}H_{39}NO_3$ | + | d-glucose |
| Veratrosine | *Veratramine, $C_{27}H_{43}NO_2$ | + | d-glucose |
| | Rubijervine, $C_{27}H_{43}NO_2$ | | |
| | Isorubijervine, $C_{27}H_{43}NO_2$ | | |

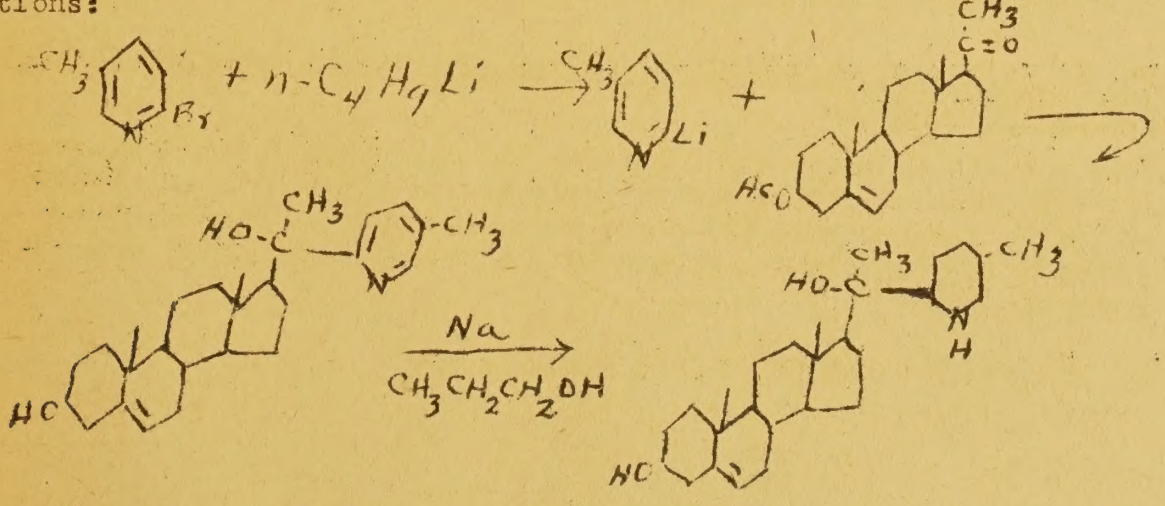
| | | | |
|---|---|---|---|
| Solasonine | *Solasodine, $C_{27}H_{43}NO_2$ | + | d-glucose, d-galactose, l-rhamnose |
| Solauricine | *Solauricidine, $C_{27}H_{43}NO_2$ | + | d-glucose, d-galactose, d-rhamnose |
| Tomatine | *Tomatidine, $C_{27}H_{45}NO_2$ | + | 2 d-glucose, d-galactose, d-xylose |
| Solanine | Solanidine, $C_{27}H_{43}NO$ | + | d-glucose, d-galactose, l-rhamnose |

*Secondary amines, others are tertiary.

The intravenous toxicity of some of these compounds is as follows:

| Alkaloid | $LD_{50}$ | |
|---|---|---|
| | mg./kg. | Micromoles/kg. |
| Protoveratrine | 0.048 | 0.06 |
| Veratridine | 0.42 | 0.63 |
| Jervine | 9.3 | 21.9 |
| Rubijervine | 70.0 | 170 |
| Cevine | 87 | 170 |
| Germine | 139 | 274 |
| Protoverine | 194 | 386 |

Dr. Uhle is interested in the synthesis of steriod secondary amines because of their cardiac activity. He has made compounds by the following type reactions:




The Chemical Nature of Fatty Acids of Bacterial Origin, by Klaus Hofmann, University of Pittsburgh.

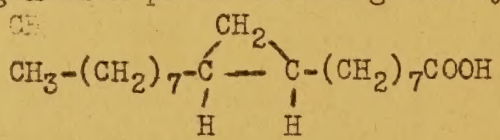
An investigation of the mechanism of action of biotin in growth production led to this present work. Williams, Louisiana State University, had

observed that fatty materials acted like biotin for L. arabinosis and casei. Later work has shown that the saponifiable fraction of the fat-soluble plasma constituents possesses biotin-like activity. Unsaturated fatty acids have been found to have biotin-like activity and oleic acid is the most active. A carboxyl group is necessary as oleyl alcohol is inactive; trans acids, such as elaidic acid, show less activity than the cis form.

Large quantities of L. arabinosis were grown and the lipid fraction collected. After saponification, methyl esters were made of the saponifiable fraction and very carefully distilled. Fractions representing $C_{16}$, $C_{18}$ and $C_{19}$ acids were collected. The largest amounts were in the $C_{16}$ and $C_{19}$ fractions. Biotin-like activity was determined on these fractions. The $C_{16}$ acid fraction had a low order of activity (0.4) as compared to the standard, oleic acid (6.0); the $C_{18}$ fraction (5.0) and the $C_{19}$ (2.4) were active. The $C_{16}$ fraction was palmitic acid; the $C_{18}$ fraction was 90% oleic acid and 10% stearic acid; the $C_{19}$ fraction was unknown.

The $C_{19}$ fraction ($C_{19}H_{36}O_2$) melted at 28-29°C, was not optically active, had two C-methyl groups, was not oxidized by $KMnO_4$ or permonophthalic acid, but absorbed 1 mole of catalytic hydrogen.

Following hydrogenation, a liquid and a solid product were separated. The solid hydrogenation product ($C_{19}H_{38}O_2$), which melted at 68-68.5°C and formed a tribromoanilide melting at 125-126°C, was identified as nonadecanoic acid. The liquid hydrogenation product ($C_{19}H_{38}O_2$), which melted at 13-14°C and formed a tribromoanilide melting at 90-94°C, was identified as a methyl octadecanoic acid. The compound which would give rise to these two hydrogenation products was given by Dr. Hofmann as



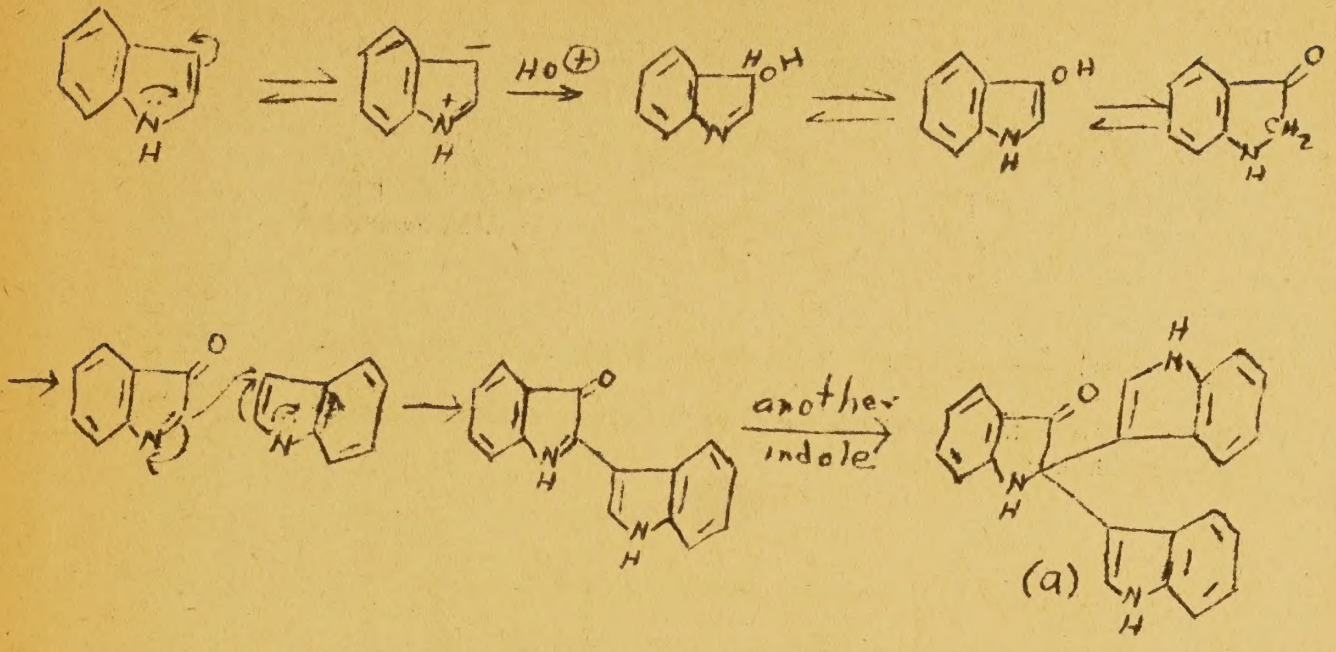
where he assumes the ring is in the middle of the chain.

On the Mechanism of Oxidation of Some Heterocylic Systems, by B. Witkop, Harvard University.

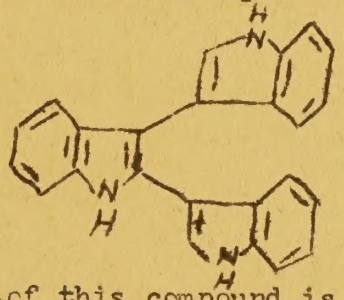
This was a very interesting presentation in which five points were covered: (1) Oxidation of simple indole derivatives; (2) Mechanism of (aut) oxidation of indole derivatives; (3) Novel demonstrations of the "medium-size ring effect"; (4) Conversion of Quinamine to cinchonamine; (5) Other rearrangements.

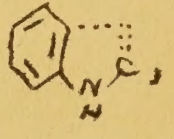
1. Oxidation of simple indole derivatives.

Schematically the oxidation of indole takes place as follows:

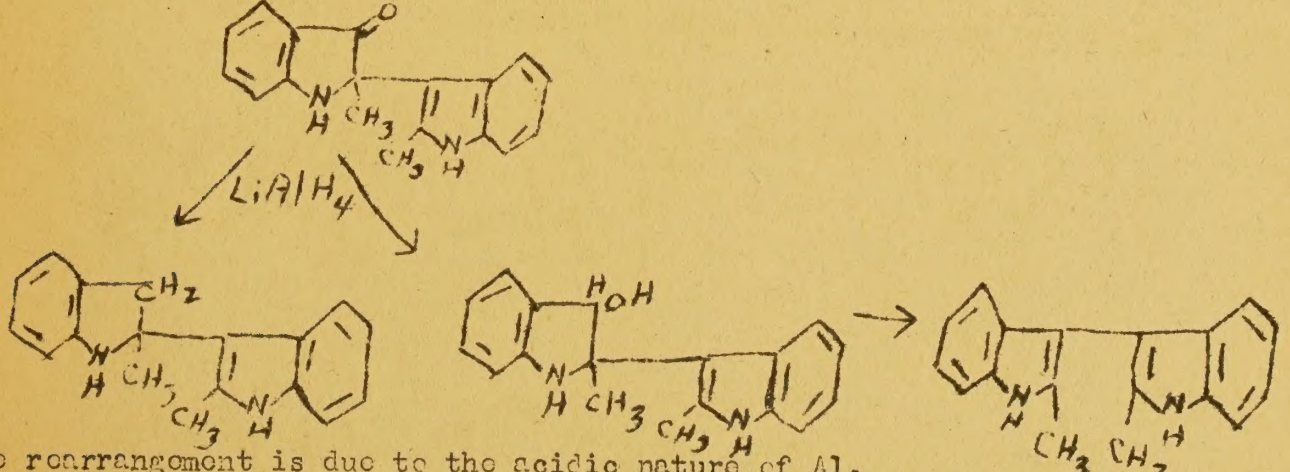



If (a) is reduced with $LiAlH_4$ a rearrangement takes place to yield

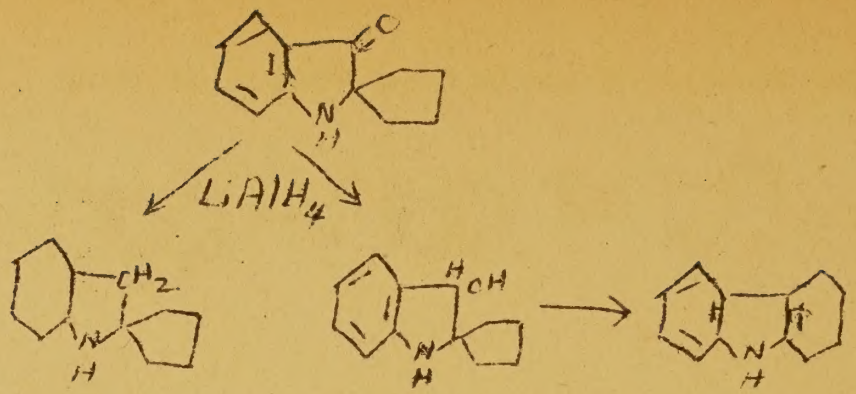


The conversion of this compound is easy to measure since the unit  has a very strong absorption at 6.1 to 6.4 microns in the infrared spectrum which is much stronger than the C=O absorption.

Other rearrangements in the presence of $LiAlH_4$ were also cited.

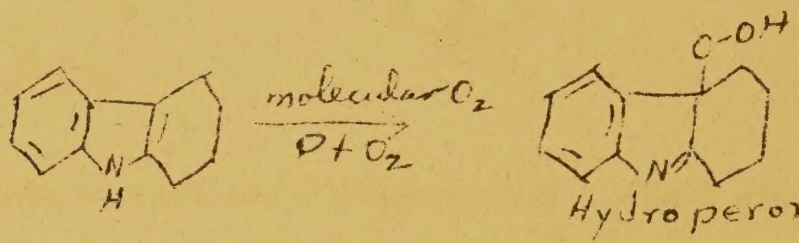



The rearrangement is due to the acidic nature of Al.



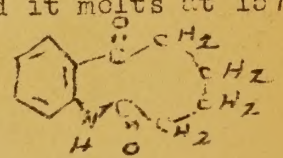

2. Mechanism of (aut) oxidation of indole derivatives.

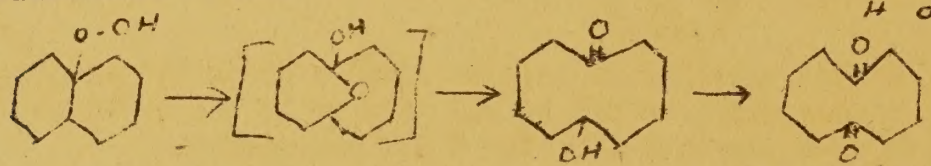
When the tetrahydrocarbazone is treated with molecular oxygen the following reaction takes place




Hydroperoxide, m.p. 159°C.

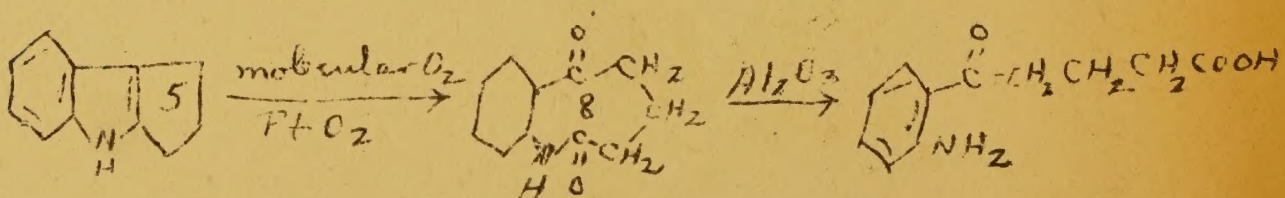
When the hydroperoxide (159°C) is recrystallized it melts at 157°C and a remarkable rearrangement has taken place. The mechanism of this reaction may be illustrated as follows:

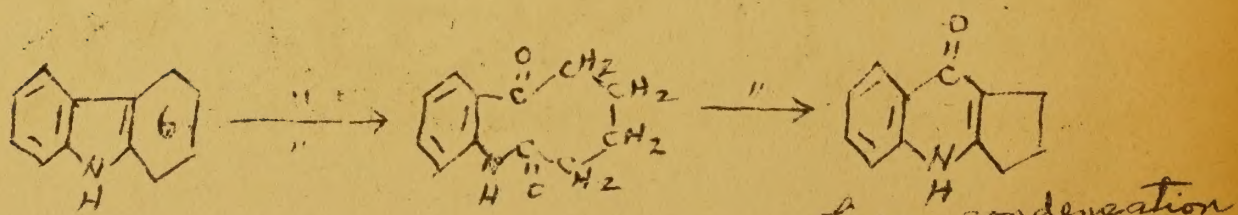


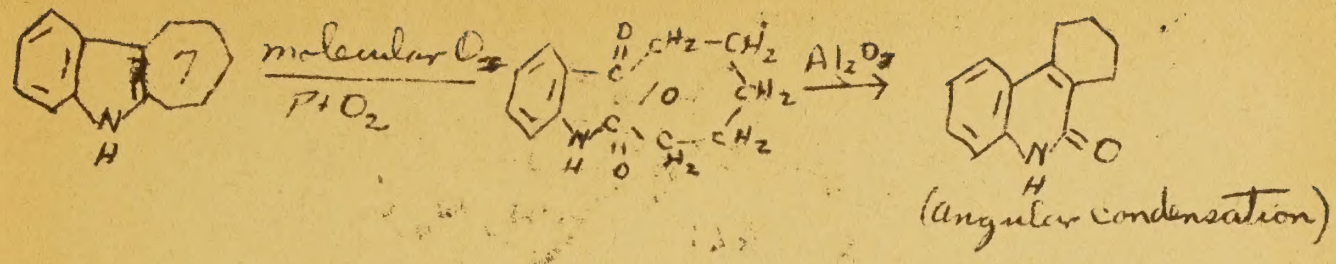



Kinetics of this change is obtained by measuring the appearance of the C=O groups in an infrared spectrometer at 5.95 - 6.0 microns.

3. Novel demonstration of the "medium-size ring effect".





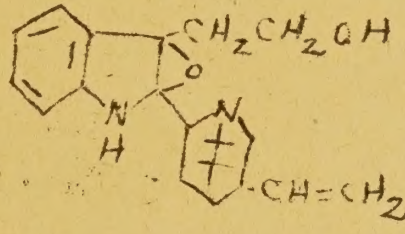

(Limiting ring size is approached here)

(Angular or linear)

4. Conversion of Quinamine to Cinchonamine.

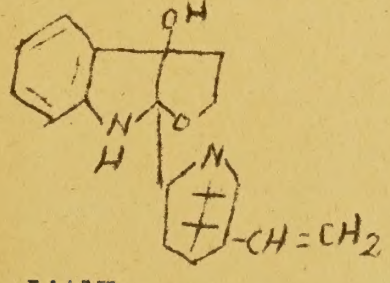
This point was the topic of another paper "The Constitution of Cinchonamine and of Quinamine", by W. I. Taylor, National Research Council, Canada, but fits into Dr. Witkops discussion.

The following structure for quinamine has been proposed by Goutarel and Taylor



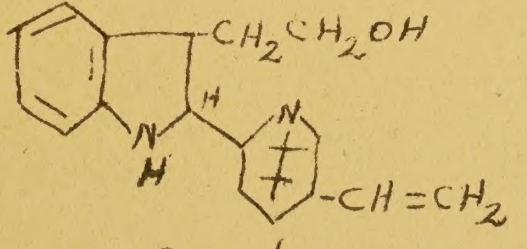
(Incorrect)

This structure was proven to be incorrect by Witkop and the following structure was assigned for quinamine



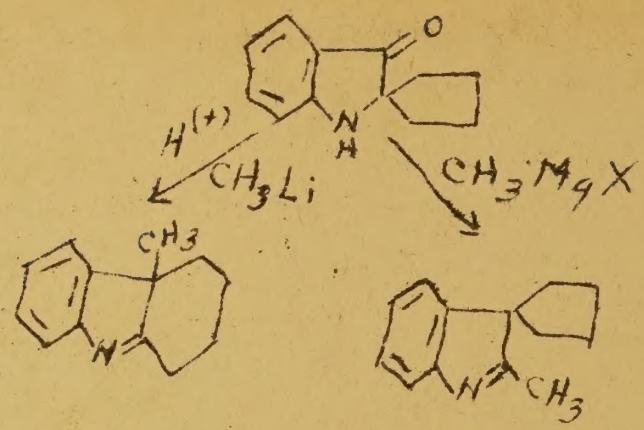
(Correct)

Quinamine $\xrightarrow{LiAlH_4}$



Cinchonamine

5. Other rearrangements.




Conclusions and Recommendations: - This seminar was limited almost entirely to the chemistry of alkaloids and related fields. New developments in these fields were reported which may influence some of the work in this Bureau.

Dr. Uhle's work on the synthesis of tomatidine, on the basis of the structural formula proposed by this Division, has progressed very rapidly but the crucial experiment involving the closing of the nitrogen ring has not been completed. Dr. Uhle very kindly agreed to keep us informed as to the progress of his work and to send us a sample of the final product for comparison with tomatidine. He stated further that there is considerable interest in the pharmacological action of steroid secondary amines.

A. E. Fontaine

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